REMARKS

In view of a Response filed 6/25/08, the Examiner has withdrawn previous prior-art rejections but entered new prior-art rejections and has made the action Final. The prosecution of this case has become circular, because the new primary reference (Sprio et al.) is simply a variant of a former primary reference (Bruchez et al.) that the Examiner ruled had been overcome (see Action of 3/25/08 at paragraph 8). In this Response, claim 37 has been amended to correct the antecedent bases in accordance with its independent claim 17.

Claim Objection

The Examiner has noted that, in independent claim 17 the term "capture probe" was amended to "hybridization probe," dependent claim 37 was not amended to conform and still recited "capture probe." Currently submitted claim 37 is amended to change "capture probe" to hybridization probe.

Decoding Distributed Arrays and the Claimed Invention

The art to which this invention relates is screening for nucleic acid target sequences in a sample utilizing arrays. Absent an array, a detection method utilizing hybridization probes and fluorophores can be for a single target or at most for a few targets (multiplexing), because emissions from only 7-8 fluorophores can be deconvolved. Detection involving a large multiplicity of targets requires using an array. Arrays can be divided into two classes: positional arrays and distributed arrays. In a positional array, a multiplicity of different hybridization (capture) probes are placed on a surface, for example, a glass slide, copies of each different hybridization probe being placed at a different, identifiable location. If a target sequence binds at a particular location, its sequence can be deduced from the location, as one knows which hybridization probe is in that location, and one knows its sequence (which is complementary to the captured target sequence). Therefore, for a positional array, there is only one detection function that must be performed, namely, to detect which hybridization probe has captured a target. In a distributed array of microcarriers, for example beads, a different hybridization (capture) probe is placed on each of numerous bead types, but the beads have no fixed location from which to discern which hybridization probe has captured a target. The claimed invention relates to distributed arrays. For distributed arrays, there are two detection functions that must

be performed. One is to detect when a target has bound to a microcarrier type. The second is to determine which microcarrier type that is – the identity of the microcarrier must be decoded in order to know which hybridization (capture) probe it carries.

The claimed invention relates to the second function – decoding beads or other microcarriers. However, we must start with the first function, capturing target sequences with the hybridization probes, because the Examiner confuses the two functions. A hybridization probe is a single-stranded oligonucleotide having a sequence that is complementary to a target sequence. It may be a conventional linear oligonucleotide. Alternately, it may be a molecular beacon probe, as specified in pending claim 37. A molecular beacon probe is a single strand that includes a central sequence complementary to a target and two flanking arm sequences that are complementary to each other. One arm carries a fluorophore, and the other arm carries a quencher. In the absence of target the arms hybridize to one another, bringing the quencher into proximity with the fluorophore. If the fluorophore is stimulated, it will not fluoresce, because energy is transferred from the fluorophore to the quencher, for example by fluorescence resonance energy transfer (FRET). When, however, a molecular beacon probe finds its target, the central sequence hybridizes to the target, which forces the arms to disengage and separates the quencher from the fluorophore, thereby causing the probe to fluoresce if the fluorophore is stimulated. Independent claim 17 recites that a microcarrier carries a hybridization probe whose hybridization can be detected. As noted, dependent claim 37 specifies that the hybridization probe is a molecular beacon probe, whose fluorescence is unquenched if a target sequence hybridizes to it.

We now turn to the critical function of decoding microcarriers. The instant application and claims recite a novel and unobvious way to code and decode microcarriers so that a hybridized target can be identified. In the prior art references relied on by the Examiner now or earlier in the prosecution of this application, that was done in one of two ways. Bruchez et al. U.S. patent No. 6,500,622, cited earlier in the prosecution by the Examiner, coded beads by impregnating them with unique combinations of differentiable fluorescent moieties, creating what is sometimes called an "optical barcode." The emission spectrum of a bead identifies its particular code. Bruchez et al. disclosed fluorescent semiconductor nanocrystals (SCNC's) as the impregnating moieties. A variant of this approach is Different SCNC's which fluoresce at different detectable wavelengths. Impregnating different bead types with different combinations

of SCNC's creates a fluorescence code that characterizes each bead and can be related to which capture probe it carries. A variant of Bruchez et al. is Spiro et al., which discloses impregnating beads with unique combinations of fluorophores rather than fluorescent SCNC's. Chee et al. (WO/2001/046675) disclosed a bead-coding method that did not require impregnating beads and, thus, did not rely on optical signatures impregnated in the beads. Chee et al. immobilized on bead surfaces a combination of unlabeled, non-fluorescent identifier oligonucleotides (not complementary to targets and, thus, not capture probes) having different sequences. After a bead had been found to have captured a target, to was subjected to a series of hybridization reactions with a series of fluorescently labeled decoder probes complementary to the identifier oligonucleotides. The fluorescence pattern from these hybridizations decoded a successful bead.

The instantly claimed invention discloses a new and unobvious way to code beads and other distributed microcarriers. Coding is achieved by immobilizing on the surfaces of beads a combination of labeled, quenched signaling hairpins that dissociate and fluoresce, thereby identifying themselves, in response to a change in condition such as temperature. An example of a signaling hairpin is depicted in FIG. 1 of this application, reproduced here:

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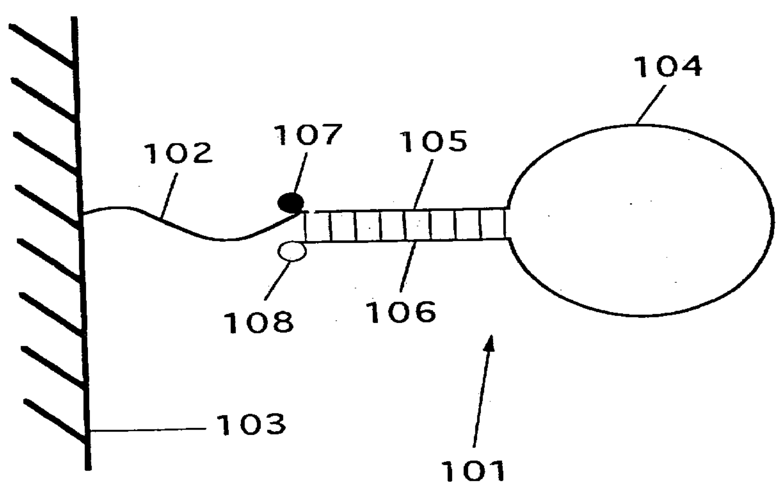

FIG. 1

The signaling hairpin 101 immobilized on a surface, as depicted in FIG. 1, is formed by a particular affinity pair, which in this case is complementary nucleotide sequences 105, 106 that are joined by linker or "linking moiety" 104, The signaling hairpin is immobilized on surface 103, for example the surface of a bead. Connected to sequence 105 is a quencher 107. Connected to sequence 106 is a fluorophore 108. Due to their proximity, quencher 107 quenches fluorophore 108, and the hairpin does not fluoresce. The affinity pair 105, 106 is disruptable by a change in a condition of its environment. For example, if the temperature is raised above the melting temperature (Tm0 of the hybrid of sequences 105, 106, the hairpin opens, the quencher is moved away from the fluorophore, and the fluorophore emits at its characteristic wavelength when stimulated. Different hairpins can have different sequence pairs with differentiable Tm's. Different hairpins can also have differentiable fluorophores. Hence, the code: combinations of different colors that appear at different prescribed environmental conditions, in this case, at different temperatures. This coding is very powerful. As explained in the instant application, if one creates a "stable" of three hairpins having different Tm's and five different fluorophores, there are 32,768 possible codes made by combinations of the hairpins. The coding scheme is also expandable by adding in another variable, namely intensity, whereby, for example, red and yellow hairpins at a ratio of 1:1 can be differentiated from the same hairpins at a ratio of 1:2. This is taught in the present application at section [0033] on page 5 of the published application.

Independent claim 17 requires that in addition to a hybridization (or "capture") probe for one of a multiplicity of target sequences, a microcarrier has a coding scheme comprising a plurality of quenched, fluorophore-labeled signaling hairpins. These hairpins are not hybridization probes for the target sequences. They have affinity pairs (stems in FIG. 1) that are disruptable by a change in environmental condition (temperature in claims 23 and 32). The disruption occurs at at least two levels. Coding is a combination of spectrally differentiable fluorophores and multiple affinity pairs (stems in FIG. 1) disruptable at detectably different levels of the condition (temperature in FIG. 1). After a bead containing temperature sensitive hairpins is found to have captured a target, it is optically decoded by changing the condition (in claims 23 and 32, temperature) and detecting fluorescence changes.

<u>Section 103 Rejection</u>

Prosecution of this application has been and continues to be, shooting at a moving prior-art target of a combination of coding references and probing references. In an Office Action dated 07/02/07, the Examiner issued a new rejection of claims then pending. All claims were finally rejected as obvious over Bruchez et al. in view of Chee et al. (a third reference, Bonnet et al. was added for dependent claims 23 and 32, which recited temperature as the changeable condition). In response, applicants filed a Request for Continued Examination (RCE) on 01/02/08, amending the claims and presenting an argument distinguishing Bruchez et al. as teaching impregnation of beads with identifiers and distinguishing Chee et al. as teaching coding with a combination of different unlabeled oligonucleotides. In the next Action of 03/25/08, the rejection based on Bruchez et al. and Chee et al. was withdrawn, but a new rejection with Stanton et al. as the primary reference was made, and certain claim terms ("capture" for the probe that binds a target, and "controllable" for the environmental condition that is varied during decoding) were found unclear. In a response filed 06/25/08, the claims were amended to their current wording to overcome the foregoing objections, and Stanton et al. was distinguished as teaching probes called aptamer beacons, which are labeled probes that bind to proteins and signal upon hybridization,

The outstanding Action of 10/22/08 comes full circle. The Examiner has withdrawn the previous rejection but entered a rejection of *prima facie* obviousness (Action, page 13) with another bead impregnation reference, previously of record, Spiro et al. (2000) *Applied and Environmental Microbiology* 66: 4258-4265 as the primary reference. Spiro et al. discloses distributed microarrays in which (1) each bead type contains a different unlabeled hybridization (capture) probe and (2) each bead is coded by being impregnated with a unique combination of two fluorescent dyes (as compared to Bruchez et al's. combinations of fluorescent semiconductor nanocrystals). As stated in the first paragraph of the right column of page 4258:

> Two classification fluorophores (in this study, red and orange dyes) are impregnated throughout the volume of the beads in varying discrete amounts, thereby creating distinct populations of "bead types" distinguishable by their red and orange intensities.

Then, under the heading **Beads** in the right column of page 4259:

> … three types of colored beads, labeled 8047, 8058 and 8059 (Luminex Corp.). These colored beads contain equal amounts of orange

> dye (no, 80) and varying amounts of red dye (no. 47, 58 and 69). A unique capture probe was attached to each bead type.

Spiro et al. is distinguishable from the claimed invention for the same reasons as Bruchez et al., as presented in the RCE of 01/02/08 and accepted by the Examiner. Although we will go through the Examiner's rejection in detail below, enough has been said already to impel withdrawal of the outstanding obviousness rejection and allowance of the pending claims.

The second reference that the Examiner relies on is Tyagi (2000) *Nature Biotechnology* 18: 597-598. Tyagi reviews a paper in the same volume of the journal, which is not relied on by the Examiner. In concluding remarks, Tyagi notes a trend away from positional arrays to distributed arrays, and restates the teaching of Spiro et al. that distributed elements [i.e., beads] in distributed arrays utilize optical barcodes:

> The optical barcodes are created by imbibing the microbeads with unique combinations of differently colored fluorescent dyes at different concentrations.

The third reference relied on by the Examiner is Bonnet et al. (1999) *PNAS* 96:6171-6176. Bonnet et al. investigates the discriminatory power of molecular beacon hybridization probes using thermodynamic principles. As noted above, a molecular beacon probe has a central sequence that is complementary to a target and flanking arms that are complementary to one another. The central sequence interacts with a target to form a probe-target hybrid (or duplex). Bonnet et al. determined that if the target is perfectly complementary to the central sequence of the probe, the probe-target duplex has a higher Tm than is the target is perfectly complementary to the central sequence except for one mismatched nucleotide. In Table 1, Bonnet et al. listed the Tm for the perfectly matched target (T:A, Tm 52.7) and the Tm's for single mismatches at different positions in the central sequence.

The fourth reference relied on by the Examiner is Landers U.S. patent No. 6,844,154. Landers discloses assays for genomic alleles (SNP's) using multiplexing and, in some cases, positional arrays. Landers does not disclose decoding microcarriers of distributed arrays. The Examiner relies on a Landers' disclosure of methods for identifying two SNP's in solution with the aid of fluorescently labeled hybridization probes, beginning at the bottom of column 15 and continuing through column 19. Each SNP site can have either of two sequences. One method involves using a pair of probes for each SNP site, one for each SNP variant. In total that is four

hybridization probes having four differentiable fluorophores. Detection involves stimulating each fluorophore and detecting its emission. A variant is to use a probes for SNP site number 1 that interact with a probes for SNP site number 2 by FRET, in which case detection is stimulating the fluorophore of a SNP 1 probe and detecting fluorescence from SNP 2 probes, which reveals haplotype.

In the rejection of independent claim 17 for *prima facie* obviousness, the Examiner concludes correctly that the first two references, Spiro et al. and Tyagi, do not teach a coding scheme for microcarriers that comprises a plurality of quenched fluorophore-labeled signaling hairpins that are not hybridzation probes, that are each an affinity pair separated by a linking moiety, wherein interaction of the affinity pair is disruptable by a change in an environmental condition, wherein disruption of one affinity pair occurs at one level of the condition and disruption of another affinity pair occurs at another level, and wherein optical decoding of the microcarriers includes changing said condition. (Action, pages 9-10). Further, the Examiner concludes correctly that Spiro et al's. coding scheme is impregnating beads with varying amounts of two different fluorophores. (Action, page 8). This is barcoding. And, the Examiner correctly concludes that Tyagi teaches barcoding that detection is to "read the optical barcode, (Action, page 8), which is just another way of saying what Spiro et al. does. The Examiner cites Tyagi on the ground that Tyagi adds to Spiro et al. the teaching to form a distributed array. That is incorrect. Spiro et al. forms a distributed array, which is just the mixture of microbeads. Tyagi is not needed for that limited purpose, and Tyagi adds nothing to Spiro et al. in that regard. That misunderstanding does not affect, however, the Examiner's correct conclusion as to what the two references together fail to show.

For the distinguishing features of claim 17, then, the Examiner must turn to Bonnet et al. This is where the Examiner confuses detection probes and signaling hairpins. As noted earlier, Bonnet et al. is a thermodynamic analysis of molecular beacon probes. See the abstract and the first paragraph:

> Molecular beacons are DNA probes with a stem-loop structure.
>
> * * *
>
> We have developed DNA probes, called molecular beacons," that become fluorescent when they bind to complementary nucleic acids.

The Examiner's interpretation "Bonnet et al. teaches [ii] a plurality of signaling hairpins that are not hybridization probes" (Action, page 10) is demonstrably wrong. Bonnet et al. does not supply what Spiro et al. and Tyagi are missing for that reason, Bonnet et al. teach hybridization probes, the other element attached to microcarriers in claim 1, not the signaling hairpins, and the rejection of claim 17 is in error for that reason alone. Table 1 of Bonnet et al. shows that hybrids formed by the central sequence of a particular molecular beacon probe and various targets have different Tm's. The legend of that figure states:

> Enthalpies, entropies, and melting temperatures for the dissociation of a perfectly complementary probe-target duplex (first entry), probe-target duplexes containing different mismatched base pairs at the same position (next three entries), and probe-target duplexes containing the same mismatched base pair at different positions (last nine entries).

The Examiner's position that Table 1 shows a number of hairpins that have different Tm's (Action, page 10) is wrong. It relates to probe-target duplexes, that is, hybrids formed between the central target-binding sequence of a molecular beacon probe and various targets, not hairpins having different Tm's.

Figure 5 of Bonnet et al. compares molecular beacon probes and linear probes for their hybridization to perfectly complementary targets and targets with a mismatch. It shows the behavior of hybrids formed with both types of targets. As stated in the legend,

> Continuous lines identify perfectly complementary probe-target duplexes and broken line identify mismatched probe-target duplexes.

The Examiner's position that Figure 5 of Bonnet teaches disruptions of different hairpins are optically differentiable is incorrect. Figure 5 teaches about probe-target hybrids, not hairpins.

Figure 2 of Bonnet et al. teaches that if a molecular beacon probe-target hybrid is heated, it will dissociate into its two components and that further heating will disrupt the probe's stem. The Examiner states that this is a teaching that by changing temperature, different signaling hairpins will be disrupted. That is incorrect. Bonnet et al. does not teach signaling hairpins or the disruption of different signaling hairpins at different temperatures.

Bonnet et al. does not supply what Spiro et al. and Tyagi admittedly fail to teach or suggest. Bonnet et al. does not teach or suggest modifying any molecular beacon probe to become a non-probe signaling hairpin. It does not teach or suggest any signaling hairpin at all. It does not teach or suggest immobilizing signaling hairpins on microcarriers. It does not teach or suggest a coding scheme of signaling hairpins. It does not teach or suggest a coding scheme

of different signaling hairpins having the same fluorophore that are disrupted at different levels of an environmental condition so as to emit differentiable signals at at least two levels of the condition.

The fourth reference relied on by the Examiner regarding claim 17 is Landers. The Examiner cites column 19 of Landers only to show how fluorophore-quenchers work. In column 19 Landers teaches that if a fluorophore and a quencher are in close proximity, FRET will occur, and there will be no emission. The Examiner infers from this that disruption of the stem taught by Bonnet et al. leads to fluorescence. That certainly is true. However, the Examiner's position that it would have been *prima facie* obvious from Bonnet et al. and Landers to use a combination of spectrally differentiable fluorophores to label a multiplicity of different affinity pairs disruptable at different levels of a condition is not supported by the references. It does not teach or suggest a code that includes the two elements of differentiable fluorophores and differential signaling at different levels of an environmental condition.

For each of the numerous reasons set forth above, the subject matter of claim 17 is not *prima facie* obvious in view of the references cited by the Examiner. The combination of references does not suggest the coding scheme of disruptable quenched, fluorophore-containing hairpins that are not probes for targets being assayed, where different hairpins are disruptable, and disrupted, differentially by changing an environmental condition.

Even if a case of *prima facie* obviousness could be made with the foregoing references, the surprising combination of features taught in this application would be sufficient to overcome *prima facie* obviousness. The combination includes:

- use of plain, non-impregnated beads
- simple and straightforward coding using oligolnucleotides synthesized on quencher (e.g., Dabcyl)-containing support columns and only a stock of a few different fluorophores to make a significant code
- a code having thousands of elements a combination of a few hairpins and a few fluorophores
- no need to use intensity to obtain a large code
- hairpin probes that can be reused by reversing the change in condition used in detection
- no hybridizations or enzymatic operations needed as part of decoding

The cited references do not suggest the foregoing combination of advantageous and desirable features.

CONCLUSION

Applicants respectfully submits that for at least these reasons the pending claims are valid and favorable reconsideration and allowance are earnestly solicited. If, however, for any reason the Examiner does not believe that such action can be taken at this time, Applicants request a personal meeting with the Examiner. The USPTO is authorized to charge Deposit Account No. 50-1943 for any charges in connection with this matter.

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Respectfully submitted,

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